

# Professional Agricultural Workers Journal

---

Volume 3

Number 1 *Professional Agricultural Workers Journal*  
(PAWJ)

12

---

9-25-2015

## Screening of Olive Cultivars for Tolerance to Fusicladium Oleagineum in South Africa

Zakhele Cyril Msimango

Department of Agriculture, Western Cape Province, Western Cape, South Africa, University of Pretoria, Pretoria, South Africa,  
[zakhelem@elsenburg.com](mailto:zakhelem@elsenburg.com)

Follow this and additional works at: <http://tuspubs.tuskegee.edu/pawj>

 Part of the [Agriculture Commons](#), [Agronomy and Crop Sciences Commons](#), [Earth Sciences Commons](#), [Horticulture Commons](#), [Other Environmental Sciences Commons](#), and the [Other Plant Sciences Commons](#)

---

### Recommended Citation

Msimango, Zakhele Cyril (2015) "Screening of Olive Cultivars for Tolerance to Fusicladium Oleagineum in South Africa," *Professional Agricultural Workers Journal*: Vol. 3: No. 1, 12.

Available at: <http://tuspubs.tuskegee.edu/pawj/vol3/iss1/12>

This Article is brought to you for free and open access by Tuskegee Scholarly Publications. It has been accepted for inclusion in Professional Agricultural Workers Journal by an authorized editor of Tuskegee Scholarly Publications. For more information, please contact [craig@mytu.tuskegee.edu](mailto:craig@mytu.tuskegee.edu).

# SCREENING OF OLIVE CULTIVARS FOR TOLERANCE TO FUSICLADIUM OLEAGINEUM IN SOUTH AFRICA

\* Zakhele Cyril Msimango<sup>1,2</sup>

<sup>1</sup>Department of Agriculture, Western Cape Province, Western Cape, South Africa

<sup>2</sup>University of Pretoria, Pretoria, South Africa

\*Email of author: zakhelem@elsenburg.com

## Abstract

This study evaluated commercial olive cultivars for resistance to olive leaf spot (OLS). A growth chamber trial was undertaken with one temperature treatment (16°C) applied during inoculation while a relative humidity (RH) of above 80% was maintained. Plants were kept for 48 hours at 99% RH after inoculation and then moved to shade netted area for disease development at a temperature of 25±5°C. The results were used for categorizing the eight olive cultivars evaluated in this study according to their OLS susceptibility. 'Frantoio' was categorized as most tolerant; 'Nandi' and 'Leccino' were found to be moderately tolerant, and 'Nocellara del Belice' fairly tolerant. 'Coratina' was found to be the most susceptible cultivar; Barouni was found to be moderately susceptible, and 'Mission' and 'Manzanilla de Seville' were found to be fairly susceptible. The findings provide a basis for preliminary recommendations of OLS tolerant cultivars and selections for commercial olive production.

**Keywords:** Cultivar Evaluation, Fungal Disease, Olive, Olive Cultivar, Olive Leaf Spot

## Introduction

Olive (*Olea europaea* sub-species *oleaceae*) trees require a Mediterranean climate-type, characterized by cold (mean monthly temperature  $\geq -3^{\circ}\text{C}$ ), wet but short winters and long, dry, hot (below  $30^{\circ}\text{C}$ ) summer conditions, which mainly occur in the Western Cape province of South Africa. Temperature is the most critical climatic factor governing olive production because olive trees have a chilling requirement for fruit bud initiation and differentiation. These conditions translate to an average temperature of  $12^{\circ}\text{C}$  in May to July (southern hemisphere), with a maximum daily temperature lower than  $21^{\circ}\text{C}$  as a minimum requirement for vegetative growth (Costa 1998). These very diverse climatic conditions are, however, favorable for the occurrence of fungal diseases, such as olive leaf spot (OLS).

Germination of the spores of *Fusicladium oleagineum* (syn. *Spilocaea oleagina*) causing OLS requires very high humidity (RH)  $\geq 80\%$ , with temperatures in a range of  $0-27^{\circ}\text{C}$ , but it is restricted at temperatures above  $30^{\circ}\text{C}$  (Obanor et al., 2010). The optimal temperature range for spore germination, infection and mycelia growth is  $16$  to  $21^{\circ}\text{C}$  (Graniti, 1993; Viruega and Trapero, 2002). Conidia develop only in the presence of free moisture or when the relative humidity is above 70% (Obanor et al., 2008; 2010). New OLS infections are associated with rainfall and mostly occur during autumn and in winter. Late winter to spring infections may stay latent (remaining symptomless) during summer. Latent lesions develop in summer during favorable temperature and humidity conditions, which serves as a source of inoculum for infection in the next season (Obanor, 2006).

Symptoms have been found to appear mainly on the leaves (Obanor et al., 2008; Sergeeva et al., 2009), usually appearing first on the upper leaf surface (MacDonald et al., 2000; Agosteo and Scalero, 2002). Numerous configurations of lesions can occur on the same leaf due to heavy infestations. Lesions start to appear as small sooty blotches of 2 to 6mm in diameter across the leaf blade. A few weeks later these blotches become muddy green to black spots that expand up to 15mm in diameter (MacDonald et al., 2000). The spots then turn dark

brown, expanding and coalescing with the adjacent infection to cover a large proportion of the leaf area. Some lesions expand to form a yellow halo that is similar to the spots on a peacock's feather, from which the names 'Peacock's spot' and 'bird's eye spot' are derived (Graniti, 1993; Obanor et al., 2008; Sergeeva et al., 2009).

Research conducted in Spain and Italy has shown that young leaves and new shoots frequently become a susceptible site of infection (Viruega and Trapero, 2002). In late winter and early spring new infections develop, leading to leaf abscission during summer, leaving partially defoliated shoots, with some healthy leaves on the tree (Costa 1998; Obanor et al., 2008; 2010). Disease infestations result in poor vegetative growth of newly developing leaves, which serves as a site of infection when conditions are favourable, which leads to twig dieback and a delay of fruit ripening (Obanor et al., 2010; 2011), and eventual yield drop. OLS is often more severe in the lower part of trees with a dense canopy.

Heavy disease infestation eventually reduces productivity and the lifespan of an olive orchard (Graniti, 1993; MacDonald et al., 2000). Graniti (1993) observed that not all infected leaves fall after infection, with the remaining infected leaves becoming a source of infection during the next season. The research shows that this pathogen can survive unfavorable conditions; that is, dry, hot weather in fallen leaves, as well as in infected leaves on the tree. Due to increasing labor costs of harvesting of olives, the worldwide olive industry including South Africa is moving towards easily mechanized planting systems (i.e., ultra-high density plantings). These new systems, however, favor disease occurrence with susceptible cultivars easily becoming infected by OLS.

The aim of the study was to assess the resistance of cultivars to fungal disease infection, specifically, OLS caused by *Fusicladium oleagineum*. The findings are expected to provide a basis for recommendation of OLS- tolerant cultivars for breeding resistance selections for commercial olive production.

## **Materials and Methods**

### **Growth Chamber Trial**

A growth chamber trial was conducted at ARC-infrutec, Bien Donne experimental farm (GPS coordinates: 33.843056°S, 18.977369°E) of Agricultural Research Council (ARC) from May 2012 to August 2012, to evaluate the tolerance of established commercial olive cultivars to OLS. The experimental layout was a completely randomized block design, with three blocks randomly allocated inside the growth chamber and within each block, 6 plants per cultivar were randomly allocated. Eight commercial cultivars ('Mission', 'Manzanilla de Sevilla', 'Frantoio', 'Nandi', 'Nocellara del Belice', 'Coratina', 'Barouni' and 'Leccino') were tested with a connotation based on a tentative grouping of cultivars by Costa (2011, unpublished) according to their susceptibility to OLS. Before inoculation (ending May 2012) the temperature in the growth chamber was adjusted to 16°C and a relative humidity (RH) of above 80% was maintained in the growth chamber for 24 h for the plants to acclimatize to the environment. After inoculation plants were kept for 48 h at 16°C and maximum RH ( $\geq 80\%$ ) and then moved to a shade net area for continued disease development after this incubation period.

### **Inoculum Production**

The inoculum used in this trial was obtained from naturally infected olive trees growing in different areas around the Stellenbosch region. Infected leaves were collected from the orchard a day before the inoculation process and stored at 4°C overnight to ensure the

viability of the spores. According to Viruega et al., (2011) the *Fusicladium oleagineum* pathogen is difficult to culture in vitro, and they found that when culturing using olive leaf extract (OLE) agar, the pathogen produced few or no conidia, thus natural infected leaves was an only option. Infected (sporulated) leaves were suspended in 300ml distilled water, and the solution was shaken at 180 rpm for 5 minutes, to dislodge the conidiophores. After filtration through Myra cloth, the inoculum solution was kept in a refrigerator (at 4°C) for 30 minutes. Thereafter, a spore count was done using a haemocytometer under the microscope (16mm magnification lenses).

### **Inoculation Process and Application of Temperature Treatments**

In mid-May 2012, six plants per cultivar were artificially inoculated by applying a conidial suspension with a concentration dosage of  $1.7 \times 10^5$  spores per ml using a manual spray. The plants were sprayed, repeatedly with the conidial suspension on the leaf surface up to incipient run-off to ensure good leaf coverage with conidial spores. The control (non-inoculated) plant material was covered with plastic bags to protect them from inoculum.

Plants were visually monitored for lesion development on a weekly basis, and disease assessments were scheduled for 6, 8, and 10 weeks after inoculation. The temperature and RH were accurately monitored using MT669 data loggers and additional mercury thermometers. Airflow from the fans was curtailed off by means of double layered nets that were hung in front of each fan, to decrease the airflow speed, as well as to prevent damage to young shoots and twigs, whilst temperature remain constant. High temperatures suppress disease development.

### **Data Collection**

Plants were monitored 14 days (2 weeks) after inoculation for lesion development; thereafter disease assessments were done every second week until week 17 after inoculation. The following measurements were taken for assessing disease incidence:

- The presence of lesions on marked leaves of experimental plants
- The disease severity on the leaf surface

Disease assessment was done using Palti's method (Palti, 1949). On each cultivar, four shoots evenly distributed around the crown were labeled. On these four shoots per plant, a position 5 to 10 cm below the shoot growing tip was marked with tape and the leaves occurring in this marked zone were monitored for lesion development. All the leaves in this marked zone were examined and appraised individually for the presence of lesions. The leaf size at assessment varied between 1 cm and 5 cm in length, depending on leaf age. Each leaf was roughly divided into four Cartesian planes for the appraisal of the necrotic areas.

On each leaf, the diameter of the lesion(s) present was measured (in millimeters) for scoring. The number of spots counted per grid was used to rank the evaluated genotypes for tolerance against OLS. Data plant leaves were numerically scored according to a six class scale (categorized as a, b, c, d, e, and f) considering the severity of leaf symptoms and the intensity of defoliation. The presence of leaf lesions was used as a measure of disease severity to group the leaves into the following categories:

- a) The leaf is free from disease (healthy);
- b) less than  $\frac{1}{4}$  of the leaf surface affected (very light infection, <24% )
- c) full  $\frac{1}{4}$  of the leaf surface affected (light infection, 25%)
- d) up to  $\frac{1}{2}$  of the leaf surface affected (moderate infection, 26-50%)

- e) up to  $\frac{3}{4}$  of the leaf surface affected (severe infection, 50-75%)
- f) over  $\frac{3}{4}$  of the leaf surface affected (very severe infection, >75%)

The degree of infection on each leaf was categorized from (a) to (f) and the number of leaves in each category was recorded separately for each type of infection. The degree of infection for each cultivar was calculated by summing the number of spots per leaf in each category. The sum of the total values obtained for all categories of leaves was then divided by the total number of examined leaves per cultivar to estimate percentage. The degree of infection calculated for each cultivar was used to rank the evaluated genotypes for tolerance to OLS disease.

A further test confirming infection was done by dipping leaves in a 5% sodium hydroxide (NaOH) solution for 2-3 min at room temperature. The 5% NaOH solution causes blemishes beneath the cuticle on the upper epidermal cells of infected leaves to become more visible, enabling visual detection of disease infection (mycelium) (Lops et al., 1993, Lopez-Doncel and Trapero, 1999) and mycelia was checked under the microscope.

### **Statistical Procedures**

Percentage disease incidence was calculated as the number of leaves with disease symptoms as a percentage of the total leaves. Percentage disease severity was calculated using the midpoint of each severity range category for individual leaves and then averaged per experimental unit. Univariate analysis of variance was performed on percentage disease incidence and severity for each assessment time separately, using General Linear Models (GLM) Procedure by employing SAS software version 9.2 (SAS Institute Inc., 1999). Observations over time were also combined in a split-plot analysis of variance with week as a subplot factor (Little and Hills, 1972). A Shapiro-Wilk test was performed on the standardized residuals from the model to test for normality (Shapiro and Wilk, 1965). Fisher's t-least significant difference was calculated at the 5% level to compare treatment means (Ott, 1998). A probability level of 5% was considered significant for all significance tests.

Logistic regression was conducted on severity scores on the last day of scoring (week 17) only, using the Logistic Procedure of SAS statistical software version 9.2 (SAS Institute Inc. 1999). Logistic regression analysis was used to investigate the relationship between the ordinal severity scores (classes) and cultivars to obtain probabilities (odds) for each cultivar relative to the severity score category estimates (class intercepts) (Hosmer and Lemeshow, 2004). Logistic regression results are illustrated using a figure that shows the relative level of infection for each cultivar to the severity score category estimates.

## **Results**

### **Visual Assessment of OLS Disease Severity**

When disease evaluation in the growth chamber began (June 2012), 20 days after inoculation (on May 2012), no visible symptoms of OLS were detected on the leaves. During the sixth week after inoculation (ending June 2012), circular black blotchy lesions became visible on the leaf surfaces. It was observed that symptoms were more visible on older leaves (from 10 weeks of age) compared to younger leaves. It is assumed that these symptoms appear after the fungus has caused internal damage to the leaves and can be seen as necrotic tissue. In this study, it was also found that the sooty blotch symptom became conspicuous while affected leaves were still on the tree.

Scoring of leaves affected by OLS was executed until leaves became detached from the plants in this trial. During week 17, detached leaves were evaluated and scored as part of this study, because according to Obanor et al., (2008) conidia of this disease can germinate on detached olive leaves when provided with free moisture and at temperatures ranging from 5 to 25°C, with an optimum of 20°C. The level of OLS infection of each cultivar is presented in Figure 1.1(a) to (f) from the first week of scoring (6 weeks after inoculation) until the end of the trial (17 weeks after inoculation).

The results presented in the six graphs in Figure 1 were obtained after a GLM procedure (t-tests) which was obtained by using SAS®9.2 statistical software, on the collected data of W6, W8, W10, W12, and until W17. The performance of each cultivar within a specific category (level of infection) throughout the evaluation period is depicted. During week 6 to week 12 after May inoculation, all cultivars were observed to have low levels of infection for all the categories (Figure 1a).

In the category  $\leq 25\%$  infection (Figure 1b), cv. ‘Nandi’ had the highest level of infection (20%) from week 6 until week 12, thereafter no new infection was further recorded for this cultivar throughout the trial. From week 12 to 17 after inoculation, an increased level of infection was found for all other cultivars, in all categories (Figure 1(a) to (f)). Based on the final visual assessment results at 17 weeks after inoculation in the category  $\leq 25\%$  infection, ‘Coratina’ was considered susceptible and ‘Frantoio’ tolerant.

In the next level of infection (category  $\leq 35\%$ , Figure 1c), ‘Coratina’ was recorded to have the highest level of infection (22%), compared to ‘Manzanilla’ (17%) and ‘Mission’ (13%). Also, in the next level of infection (category  $< 50\%$ , Figure 1d), Coratina’ (16%), ‘Mission’ (12%), and ‘Manzanilla’ (11%) had higher levels of infection, while ‘Nocellara del Belice’ had lower levels of infection, less than 10% infection.

In category  $\leq 75\%$  (Figure 1e), ‘Manzanilla’ (8%) had a higher level of infection compared to ‘Coratina’ (6%), and ‘Mission’ (3%). At the maximum level of infection ( $> 75\%$ , Figure 1f), ‘Mission’ had the highest level of infection (7%) while ‘Coratina’ had 2%, and none of the other cultivars were observed to have this level of infection. It was observed that this high level of leaf infection resulted in defoliation, as heavily infected leaves were observed on the ground. It was also noted that most of the infected leaves appeared to fall prematurely when infection was high.

Table 1 depicts the OLS severity calculated for the eight olive cultivars evaluated at 17 weeks after inoculation. At present, there is no standard minimum value (threshold value) for OLS infection that can be used for comparison between cultivars. It still needs to be established through research. In the healthy leaf category, ‘Frantoio’ and ‘Nandi’ had the lowest level of infection with 85% and 83% healthy leaves, respectively. In the category  $\leq 25\%$  infection, cvs ‘Frantoio’ and ‘Nandi’ had the lowest level of infection with 10% and 8%, respectively. Only the cv. ‘Coratina’ had a significantly higher level of infection (24%) compared to the other cultivars evaluated. In the next category ( $\leq 35\%$  infection), ‘Coratina’ again had the highest level of infection (20%), followed by ‘Manzanilla’ (17%) and ‘Mission’ (13%). In the category  $\leq 50\%$  infection, the level of infection of cvs ‘Barouni’, ‘Coratina’, ‘Mission’, and ‘Manzanilla’ had significantly higher levels of infection than those of the other four cultivars evaluated. They were, respectively, 16%, 16%, 11%, and 12%. There were no significant differences in the  $\leq 75\%$  infection category between cultivars. In the category high infection ( $> 75\%$ ), cvs ‘Mission’ and ‘Barouni’ had the highest levels of infection at 7% each.

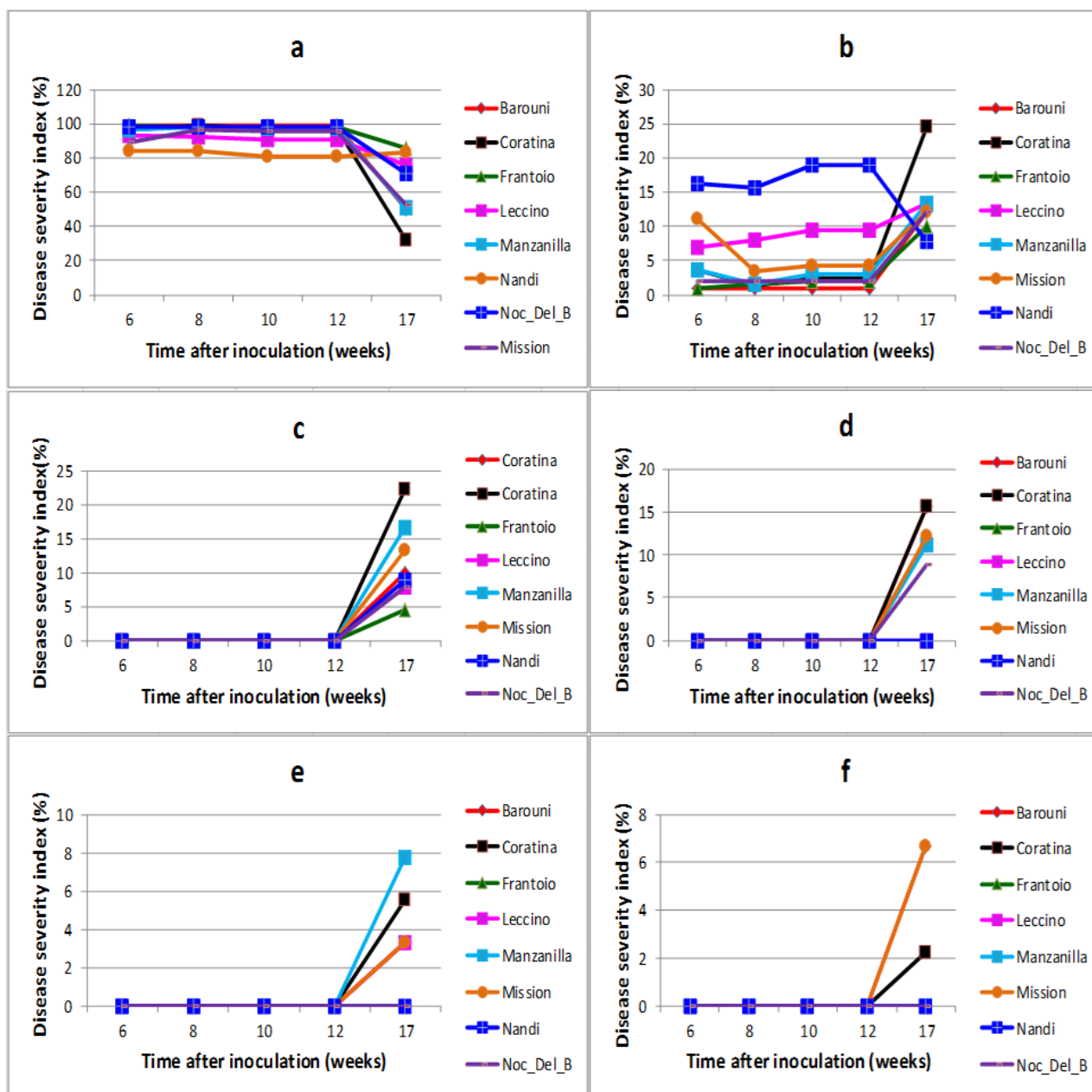


Figure 1. The tolerance of the eight cultivars to OLS disease during the growth chamber trial (2011/12 season) observed in (a) healthy leaves; (b)  $\leq 25\%$  infection of the leaf surface; (c)  $\leq 35\%$  infection of the leaf surface; (d)  $\leq 50\%$  infection of the leaf surface; (e)  $\leq 75\%$  infection of the leaf surface; and (f)  $> 75\%$  infection level of the leaf surface

### Laboratory Test to Confirm Results of Visual Assessment

During the 17<sup>th</sup> week after inoculation, laboratory tests were performed on 100 infected leaves of each commercial cultivar. The laboratory test was performed to expose visually undetectable symptoms and confirm infection level on the leaves. The scoring of leaves, during the laboratory test, was used to rank each cultivar for tolerance against OLS disease.



Table 1. Mean OLS Severity Calculated for Eight Olive Cultivars 17 Weeks after Inoculation with *F. oleagineum* in the Growth Chamber Trial at Bien Donne (2011/12 seasons). This was tested at the 0.05 level of significance.

Cultivars	<i>Level of infection coverage per 100 leaves</i>					
	healthy leaf (significant difference)	≤25% Infection (significant difference)	≤35% Infection (significant difference)	≤50% Infection (significant difference)	≤75% Infection* (no significant difference)	>75% Infection (significant difference)
<b>Barouni</b>	52 <i>bcd</i>	12 <i>b</i>	10 <i>bc</i>	16 <i>a</i>	3 <i>a</i>	7 <i>a</i>
<b>Coratina</b>	32 <i>d</i>	24 <i>a</i>	20 <i>a</i>	16 <i>a</i>	6 <i>a</i>	2 <i>ab</i>
<b>Frantoio</b>	86 <i>a</i>	10 <i>b</i>	4 <i>c</i>	0 <i>b</i>	0 <i>a</i>	0 <i>b</i>
<b>Leccino</b>	76 <i>ab</i>	13 <i>b</i>	8 <i>bc</i>	0 <i>b</i>	3 <i>a</i>	0 <i>b</i>
<b>Manzanilla</b>	51 <i>bcd</i>	13 <i>b</i>	17 <i>ab</i>	11 <i>a</i>	8 <i>a</i>	0 <i>b</i>
<b>Mission</b>	53 <i>bcd</i>	12 <i>b</i>	13 <i>abc</i>	12 <i>a</i>	3 <i>a</i>	7 <i>a</i>
<b>Nandi</b>	83 <i>a</i>	8 <i>b</i>	9 <i>bc</i>	0 <i>b</i>	0 <i>a</i>	0 <i>b</i>
<b>Noc. del Belice</b>	71 <i>abc</i>	12 <i>b</i>	8 <i>bc</i>	9 <i>ab</i>	0 <i>a</i>	0 <i>b</i>

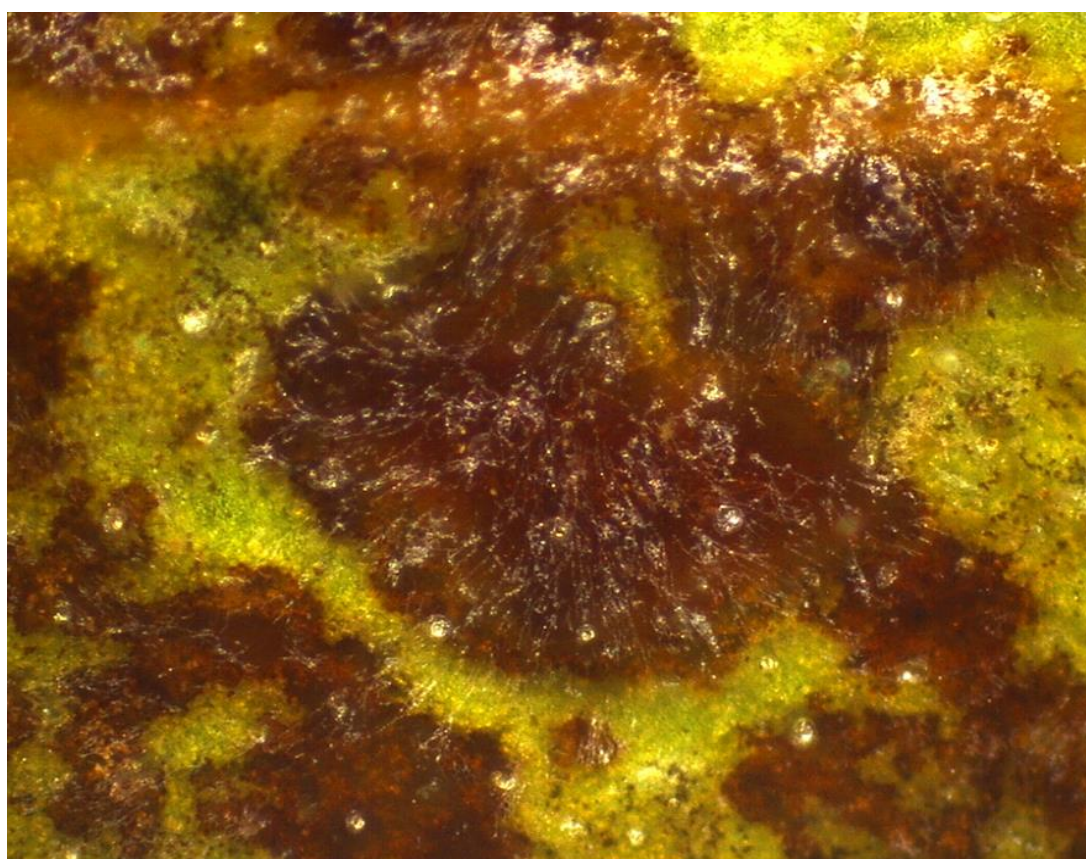


Figure 2. Blemishes beneath the cuticle on the upper epidermal cells of a heavily infected leaf after dipping leaf into 5% NaOH solution



The 5% NaOH solution caused blemishes beneath the cuticle on the upper epidermal cells of infected leaves (Figure 2) indicating the presence of mycelia and enabling visual detection of disease infection. In Figure 2, a fine whitish hair-like network (hyphae of the mycelium) is visible inside the leaf tissue as detected under a light microscope (16mm magnification lens) using lactophenol blue solution (one drop).

The class intercepts that were obtained from the logistic regression analysis together with the least significant difference (LSD) means of the infection locality from the ANOVA were used to assess the level of susceptibility of each cultivar. Cultivars ‘Frantoio’, ‘Nandi’, ‘Leccino’, and ‘Nocellara Del Belice’ which intercepted in the same block below the healthy line (black line) showed significant tolerance to OLS disease infection, while ‘Coratina’, ‘Barouni’, ‘Mission’, and ‘Manzanilla’ intercepting above the healthy line showed susceptibility (Figure 3).

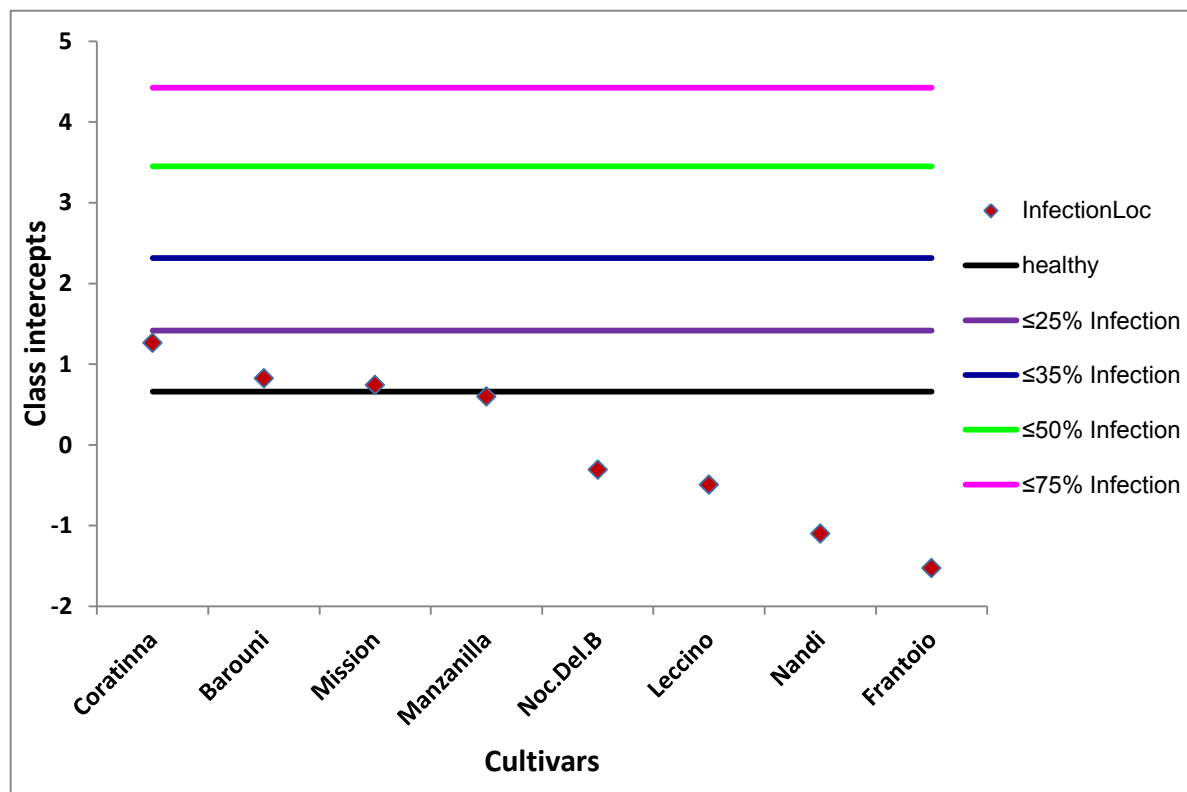


Figure 3. Infection location (InfectionLoc) identifying the level of susceptibility for eight olive cultivars using the logistic regression analysis during Week 17 for the trial at Bienne Donne

## Discussion

### Disease Development

After a month of inoculation in the growth chamber, there were no visible symptoms of OLS on the leaves. During the 12<sup>th</sup> week after inoculation OLS symptoms began to be visible as black circular spots on the leaf surface. The affected area on the leaf blade formed chlorotic circles around necrotic blotchy lesions. The infection became more prominent and conspicuous while infected leaves were still attached to the trees. Furthermore, it was found that the symptom development was easier to observe on the older leaves (at least 10 weeks of age) than on younger leaves. These findings are similar to those of Obanor et al. (2011) who showed that the age of olive leaves significantly affects conidia germination and development. Disease infection seemed to occur at an early stage while the leaves are tender and less cutinized but as they get older, spore germination decreases and more spores are produced for survival. Similar results have been reported for some other pathogens (Bentes and Matsuoka 2002). The black circular spots are an indication of production of conidia to overcome unfavorable surrounding climatic conditions. Results from this current study gave evidence that disease onset and infection occurs at an early stage while leaves are still young, although visual symptoms appear at a later stage (10 weeks after inoculation). Disease severity was correlated with leaf age with the highest severity observed on old leaves (12 weeks) when leaves showed necrotic symptoms (senescing appearance).

The laboratory test using 5% NaOH solution confirmed infection on the leaves. Apart from the visual symptoms associated with OLS that were detected during the visual assessment of leaves, “white spot” symptom was also found, and ‘Nandi’ had the highest incidence of this symptom. Further investigation is needed to find its morphology and causal agent. However, the occurrence of the white spot symptom did not continue, and there was no significant damage to the plants.

The results of the growth chamber trial were used for final categorizing of the eight olive cultivars evaluated in this study according to their OLS susceptibility (Table 1). ‘Coratina’ was found to be the most susceptible cultivar (highly susceptible); ‘Barouni’ was found to be moderately susceptible. Two of the well-established and important cultivars grown in the South African olive industry, ‘Mission’ and ‘Manzanilla de Seville’ were also found to be fairly susceptible. ‘Frantoio’ was categorized as highly tolerant, while ‘Nandi’ and ‘Leccino’ were found to be moderately tolerant, and ‘Nocellara del Belice’ was fairly/slightly tolerant. This agrees with the findings by Costa (1998) who stated that all eight cultivars evaluated in this study are susceptible to OLS under South African conditions.

This study’s results contradict the results indicated by Costa (1998) that cv. ‘Coratina’ is fairly resistant. ‘Coratina’, ‘Barouni’, ‘Mission’, and ‘Manzanilla’ were found to be susceptible cultivars under South African conditions in this study. In California, Sutter (1994) found that ‘Manzanilla’, ‘Barouni’, and ‘Mission’ were among the most tolerant cultivars against OLS disease. The results of this study also contradict the findings of Mekuria et al. (2001) that ‘Manzanilla’ was semi-resistant under field conditions. This could be related to cultivar’s gene adaptability to a specific region (e.g., ‘Manzanilla de Seville’ and ‘Manzanilla de Jean’ (Spain), with suffix “de Sevilla, etc.” means country of its origin).

A possible clarification by Obano et al. (2008) could be that either the tree was able to adapt differently to a specific climate conditions or a particular variety differs in levels of epicuticular wax, and thus, different level of chemicals in various cultivars could have contributed to resistance against the disease. In a similar study conducted in Spain, Viruega

and Trapero (1999) found that under field conditions disease occurrence varied between seasons; consequently, further studies are underway.

### **Grouping of Cultivars According to their OLS Susceptibility**

Apart from the work of Costa (1998) and the tentative grouping of commercial olive cultivars and selections according to their levels of susceptibility to OLS by Costa (2011), no other published research results regarding OLS susceptibility of olive cultivars under South African conditions are available. The findings of the study provide a basis for further evaluation of OLS-tolerant cultivars and selections both in a controlled environment and under field conditions. The findings also provide a basis for preliminary recommendations of OLS tolerant cultivars and selections for commercial olive production. The choice of cultivar presumably depends to a large extent on the climatic conditions of a specific area. However, further investigation is needed to assess the risk of OLS disease occurrence for established, as well as new olive growing regions of South Africa, as they have different climate conditions.

The Paarl area, where the South African olive industry is concentrated at present, is characterized by favorable climatic conditions for the occurrence of OLS. Winter temperatures can fall to a minimum of less than 4°C, with a maximum of approximately 18°C. Average temperatures can range between a minimum of 10°C to a maximum of 15°C and 84% maximum RH during the autumn/winter season, as observed in the 2011, 2012, and 2013 seasons. This type of condition, plus very wet weather, favor OLS disease occurrence. Therefore, based on OLS tolerance, ‘Frantoio’ (highly tolerant), ‘Nandi’ and ‘Leccino’ (moderately tolerant), and ‘Nocellara del Belice’ (fairly tolerant) are considered well-adapted cultivars for commercial production in the Paarl area. ‘Coratina’ (highly susceptible), ‘Barouni’ (moderately susceptible), and ‘Mission’ and ‘Manzanilla de Seville’ (fairly susceptible) are expected to be more prone to be infected by OLS in the Paarl area, with the consequent negative effects on growth and yield.

When comparing the Paarl area to some new olive planting areas such as Riebeeck-West (newly important olive producing area) which is characterized by high RH conditions (above 70% during winter), disease occurrence is likely to be a problem in this winter rainfall area. In specific summer rainfall areas the incidence of OLS disease could be less of a problem, due to the fact that dry (little rain, low relative humidity), warm weather conditions with temperature above 27°C suppress spore germination. Oudtshoorn and Prince Albert in the Klein Karoo, as well as Vaalharts and Barkley-West in the Northern Cape are characterized by drier and warmer conditions, which are less favorable for the occurrence of OLS compared to the Paarl area. Therefore, based on OLS tolerance, ‘Coratina’ (highly susceptible), ‘Barouni’ (moderately susceptible), and ‘Mission’ and ‘Manzanilla de Seville’ (fairly susceptible) are expected to be suitable cultivars for commercial production in these areas, provided that other climatic requirements for commercial production are met.

Summer rainfall areas that experience warm winter conditions and high temperatures in summer could be detrimental to OLS disease development. Dry weather conditions (Karoo) and very warm conditions are detrimental to disease build-up and development, and thus under these conditions the disease may be kept in a latent stage for a long time until a future outbreak. In this case, alternative control measures are important, and tolerant cultivars could be used to manage OLS disease. Previous research stated that disease severity increases with increased temperature from 5°C to 15°C and then decreases from 15°C to 25°C, whereas spores die above 27°C (Viruega et al., 2011). Currently, tolerant cultivars could contribute to minimizing chemical input and reducing disease incidence.

## Conclusion

With reference to OLS tolerance, 'Coratina' was found to be a highly susceptible cultivar; 'Barouni' (moderately susceptible), and Mission and 'Manzanilla de Seville' were fairly susceptible. These cultivars are among the most important cultivars in the South African olive industry. However, for high-density planting systems, using susceptible cultivars could require more chemical inputs to control fungal diseases such as OLS. Therefore, it is recommended that OLS tolerant cultivars be used for high-density planting systems. Based on OLS tolerance, 'Frantoio' (highly tolerant), 'Nandi' and 'Leccino' (moderately tolerant), and 'Nocellara del Belice' (fairly tolerant) are considered well-adapted cultivars for commercial production for all olive growing regions of South Africa. These cultivars are recommended for use in breeding of new selections for OLS tolerance. The results of the study were obtained over one season only (2011/12). It is, therefore, recommended that the evaluation of OLS tolerance of the eight cultivars included in this study should be repeated over more than one season, both under controlled conditions and field conditions in the major olive production regions of South Africa to verify the results.

## Acknowledgements

I wish to express my sincere gratitude and appreciation to the Department of Agriculture: Western Cape Government for funding my studies and the entire project, as well as for hosting me during the research. Also, special thanks go to the Agricultural Research Council (ARC) Infruitec-Nietvoorbij, Stellenbosch, Bien Donne Experimental Farm for allowing me to use their facilities.

## References

- Agosteo, G.E., and L. Scolaro. (2002). "White Spots: An Atypical Symptom of Olive Leaf Spot." *Journal of Plant Pathology* 84 (3): 171 – 200.
- Bentes, J.L.S., and K. Matsuoka. (2002). "Histology of *Colletotrichum guaranicola* and *Paullinia cupana* var. *sorbilis* on Resistance and Susceptible Clones." *Fitopatologia Brasileira* 27: 71-77.
- Costa, C. (1998). *Olive Production in South Africa, A Handbook for Olive Growers*, Stellenbosch, South Africa: ARC-Infruitec.
- Costa, C., (2011). Tentative Grouping of Commercial Olive Cultivars in South Africa (unpublished). Agricultural Research Council (ARC) Infruitec-Nietvoorbij, Stellenbosch, South Africa
- Graniti. A. (1993). "Olive Scab: A Review." *Bulletin OEPP/EPPO*, Bulletin 23: 377-384.
- Hosmer, D.W., and S. Lemeshow. (2004). *Applied Logistic Regression*, 2<sup>nd</sup> ed. John Wiley and Sons.
- IOC (International Olive Council). (2013). "Olive Oil Imports in 2012/13." *Market Newsletter* December, (78): 1-4.
- Little, T.M., and F.J. Hills. (1972). *Statistical Methods in Agricultural*. Davis, CA: University of California, Davis.
- Lopez-Doncel LM, and A. Trapero. (1999). "Resistance of Olive Tree Cultivars to Leaf Spot caused by *Spilocaea oleagina*." *Proceedings of the 3<sup>rd</sup> International Symposium on Olive Growing, Acta Horticulturae* 474: 549-553.
- Lops, F., S. Frisullo, and V. Rossi. (1993). "Studies on the Spreading of the Olive Scab Pathogen *Spilocaea oleagin*, *OEPP/EPPO Bulletin* 23: 385-387.
- MacDonald, A.J., M. Walter, M. Trought, C.M. Frampton, and G. Burnip. (2000). "Survey of Olive Leaf Spot in New Zealand." *New Zealand Plant Protection* 53: 126-132.

- Mekuria, G.T., G. Collins, and M. Sedgley. (2001). "Identification of Genetic Markers in Olive Linked to Olive Leaf Spot Resistance and Susceptibility." *Journal of American Society of Horticultural Science* 126 (3): 305-308.
- Obanor, F.O. (2006). "Olive Leaf Spot: Epidemiology and Control" PhD Thesis, Ecology Department, Faculty of Agriculture and Life Sciences, Lincoln University, Canterbury, New Zealand.
- Obanor, F.O., M. Walter, E.E. Jones, and M.V. Jaspers. (2011). "Effect of Temperature, Inoculum Concentration, Leaf Age, and Continuous and Interrupted Wetness on Infection of Olive Plants by *Spilocaea oleagina*." *Plant Pathology* 60: 190-199.
- Obanor, F.O., M. Walter, E.E. Jones, M.V. Jaspers. (2010). "Effect of Temperature, Inoculum Concentration, Leaf Age and Continuous and Interrupted Wetness on Infection of Olive Plant by *Spilocaea oleagina*." *Plant Pathology* 59: 1365-3059.
- Obanor, F.O., M. Walter, E.E. Jones, M.V. Jaspers. (2008). "Effect of Temperature, Relative Humidity, Leaf Wetness and Leaf Age on *Spilocaea oleagina* Conidia Germination on Olive Leaves." *European Journal of Plant Pathology* 120: 211-222.
- Ott, R.L. (1998). *An Introduction to Statistical Methods and Data Analysis*. Belmont, CA: Duxbury Press.
- Palti J. (1949). "Methods of Assessing the Incidence of Olive Leaf Spot: A Contribution to the Technique of Plant Disease estimation." *Plant Pathology* 156-166.
- SAS Institute Inc., (1999). *SAS/STAT User's Guide*, Version 9, 1st printing, Volume 2, SAS Institute Inc., SAS Campus Drive, Cary, NC.
- Sergeeva, V., U. Braun, R. Spooner-Hart, N.G. Nair. (2009). "Observation on Spot caused by *Fusicladium oleagineum* on Olives (*olea europaea*) in New South Wales, Australia." *Australian Plant Disease Notes* 4: 26-28.
- Shapiro, S.S., and M.B. Wilk. (1965). "An Analysis of Variance Test for Normality (Complete Samples)" *Biometrika* 52: 591-611.
- Sutter, E.G. (1994). "Olive Cultivars and Propagation." In L. Ferguson, G.S. Sibbett, and G. Martin. (eds.), *Olive Production Manual* (pp. 23-25). Oakland, CA: Division of Agriculture and Natural Resources.
- Viruega, J.R., and A. Trapero. (1999). "Epidemiology of Leaf Spot of Olive Tree caused by *Spilocaea oleagina* in Southern Spain." *Acta Horticulturae* 474: 531-534.
- Viruega, J.R., and A. Trapero. (2002). "Effect of Temperature, Wetness Duration and Leaf Age on Infection and Development of Olive Leaf Spot." *Acta Horticulturae* 586: 797-800.
- Viruega, J.R., L.F. Roca, J. Moral, and A. Trapero. (2011). "Factors Affecting Infection and Disease Development on Olive Leaves Inoculated with *Fusicladium oleagineum*." *The American Phytopathological Society* 95 (9): 1139-1146.
- Wilson, E.E., and H.N. Miller. (1949). "Olive Leaf Spot and its Control with Fungicides." *Hilgardia* 19: 1-24.